Phytochemical Methods for the Extraction and Analysis of Seed Coat Flavonoids from Common Bean (Phaseolus vulgaris L.)

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Introduction

The expression and inheritance of seed coat color in *Phaseolus vulgaris* L. has been the subject of study since early in this century. Despite advances that have been made, we still have not identified the phenolics and flavonoids that make up seed coat color for known genotypes. In his synthesis of the possible genes controlling seed coat color in *P. vulgaris*, Leakey (1988) has proposed a scheme that outlines the genes and possible pathways for the biosynthetic conversion of simple flavanones to the flavonols and anthocyanins that are responsible for seed coat color. As part of our research to identify these compounds in the seed coat of *P. vulgaris* we were interested in examining several genetic stocks with known seed coat color. We obtained ten genetic lines from M. Bassett (University of Florida, Gainesville, FLA). The seed coat colors of the genetic stocks are known as: mineral brown (MB), mat soft brown (MSB), yellow brown (YB), buffy citrine (BC), dark brown violet (DBV), mat black (MTB), grey white (GW), 5-593, VO400 and VOO59. Except for VO400 and VOO59 the stocks are different from each other by one or more recessive allelic substitutions at the color determining loci, and were developed with the Florida dry-bean breeding line 5-593 as the source of the dominant alleles. Line 5-593 has bishop's violet flowers, determinant growth habit, small and shiny black seeds with the seed coat genotype *T, P^{gri}, [Cr], D, J, G, B,V, Rk* (Bassett 1994b, 1996). For several years Bassett (1992, 1994a) has been developing key genetic stocks for seed coat color in order to study the genetics of color inheritance in accessions with an unknown genotype Bassett.

The purpose of our study was to provide other investigators with methods for the extraction and isolation of secondary compounds, in particular flavonoids, from the seed coat of *P. vulgaris*. We have found that the procedures we employed were relatively simple and resulted in good recovery of flavonoids for analysis.

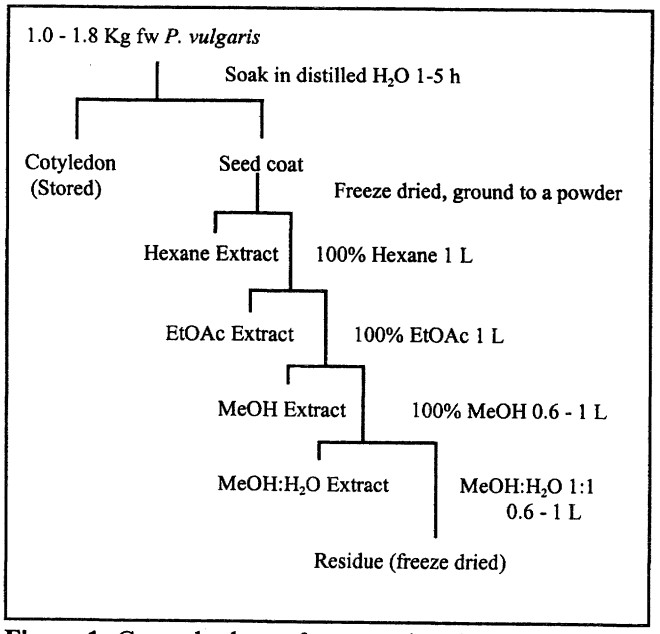

Figure 1: General scheme for extracting flavonoids from *P. vulgaris* seed coat.

Materials and Methods

Prior to extracting flavonoids from seed coats, we needed first to separate the seed coats from the cotyledons because we found (similar to Feenstra 1960) that extraction of the whole bean did not yield color compounds. This may be due to the seed coat phenolics and anthocyanins binding to the starch and proteins of the cotyledon. An additional problem we faced was that because seed coat pigments are localized exclusively in the lumen of the epidermal cells, these compounds constitute a low percentage of the dry weight of seed coats (Feenstra 1960). We therefore soaked and de-corticated 1-1.8 Kg of whole bean to give at least 100 g of dried seed coat for extraction (Fig. 1) (Beninger et al. unpublished data). Water exudate from the soaking was freeze-dried and

stored for future analysis. Dried seed coat (100 g) was then loaded into a glass column (2" diam X 12" height) and extracted sequentially with hexane, EtOAc, MeOH and MeOH:H_2O 1:1 (Fig. 1). Extracts were dried under reduced pressure in a rotary evaporator and stored at -20 C. Samples from each genetic line were then spotted on a silica thin layer chromatography plate (TLC) (solvent system $CHCl_3$:MeOH 4:1) (Fig. 2).

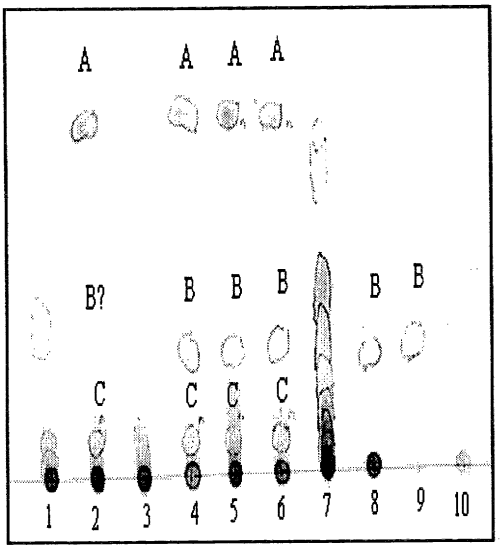

Figure 2: Preliminary TLC analysis of *P. vulgaris* seed coat flavonoids. 1-mat black, 2-mineral brown, 3-dark brown violet, 4-yellow brown, 5-mat soft brown, 6-buffy citrine, 7-5-593, 8-VO59, 9-VO400, 10-grey-white. A,B &C discussed in text.

Results and Discussion

Recoveries averaged 0.2% for the hexane, 0.31% EtOAc, 9.8% MeOH and 6.72% MeOH:H_2O fractions respectively. Recoveries therefore averaged 17% all fractions combined. A preliminary TLC of the ten genetic lines showed a number of phenolics and flavonoids (Fig. 2). Compound A was common to MB, MSB, YB, and BC. Compound C was also present in these four genotypes. Compound B was present in MSB, YB, and BC but appeared faint in MB on the TLC shown. Further examination has shown B to also be present in MB. At least three anthocyanins are present in DBV, MTB and 5-593. Compound B is also present in VOO59 and VO400, but is absent in the anthocyanin containing lines MTB, DBVand 5-593. Compounds A and B were also absent in the MTB, DBV and 5-593 lines. The genetic line GW does not appear to contain any anthocyanins or flavonoids. Compound A has been further fractionated and purified and is undergoing tests such as hydrolysis, UV with shift reagents, ^{1}HNMR and ^{13}C to determine its structure. In addition, compounds B and C and anthocyanins present in MTB, DBV, and 5-593 are currently undergoing purification.

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